Environmental Health Perspectives
Vol. 61, pp. 321-328, 1985

QSAR Analysis and Data Extrapolation among Mammals in a Series of Aliphatic Alcohols

by Miloň Tichý*, Václav Trčka,† Zdeněk Roth,* and Marie Krivucová*

Concepts of QSAR analysis and biological similarity models are combined for use in extrapolation of LD_{50} values after IP application of a series of aliphatic alcohols (C_1–C_5) to mouse, hamster, rat, and guinea pig and rabbit. It has been found that although close correlation exists between LD_{50} values after IP and IV applications for mouse and rat, the QSARs obtained with LD_{50} after IV application are not suitable for a prediction of LD_{50} values after IP application for rabbit. Different transformation or distribution processes in mouse, rat, and rabbit after the two types of applications might be the reason.

The LD_{50} values (expressed in mmole/m^2 of body surface) seem to be independent of mammalian species used (at least within the mouse, rat, hamster, and probably guinea pig series). This fact makes it possible to predict reasonable values of LD_{50} after IP application for rabbit. Expression of toxicity in mmole/m^2 of body surface may be useful in toxicological studies.

The model of quantitative structure–activity–species relationships (QSASR) for the system of alcohols and animals chosen is proposed:

$$\log BA_{ij} = k_j + l_j \log X_i$$
$$\log BA_{ij} = a_j + b_i \log Z_j$$

where i denotes an alcohol, j an animal, BA being LD_{50} (mmole/m^2) after IP application, X molecular connectivity $^1\chi$ and Z body surface: body weight ratio. The model is based on the assumption that b_i is independent of chemical structure (being zero or close to zero), a_i is a function of molecular connectivity $^1\chi$, k_j and l_j being independent of animal species. These assumptions resulted from the statistical analysis of QSARs and allometric equations obtained under various conditions.

Introduction

An enormous effort has been devoted to solving the problem how to extrapolate data obtained on one animal to another animal or even to man. The results of a number of tests on biological models and experimental animals are extrapolated to man mostly taking into account relative differences in body weight or in body surface on the supposition that man reacts similarly to the model.

For a description of differences in physiological functions among various species, empirical allometric equations were suggested based on a biological similarity model (*1–4*). The toxic responses of several toxicants studied as a quantitative function of body weight within one animal species demonstrate that weight may be used for the extrapolation of data on toxic tests from one size animal to another size (*5*). It was reported (*6,7*) that the relationship between response and dose can be best expressed when the independent variable is plotted as a total amount given each animal. Other investigators advocated that the dose should be corrected by a two-thirds power of body weight ("surface area factor"). This factor proved to be useful, e.g., for a prediction of lethal toxicity of antineoplastic agents for different size animals not only within, but also among several mammalian species (*8*). The following equation is in accordance with the proposition (*9*) to relate dosage to an exponent of body weight that need not be necessarily two-thirds:

$$Y = a + b \log M/W^h \quad (1)$$

where Y is survival time after dosage M of sodium arsenate ingested by silkworm larvae of different size and development, W represents some measure of body size, h an exponent that can be defined as a ratio of regression

*Institute of Hygiene and Epidemiology, 100 42 Praha 10, Czechoslovakia.
Research Institute for Pharmacy and Biochemistry, 130 60 Praha 3, Czechoslovakia

slopes of the relationships between the response and the quantities of dose and body weight (*9*).

Equation (2) represents the customarily used formula:

$$\log C = \log a + b \log W \quad (2)$$

showing a linear relationship between log of body weight (W) and LD_{50} or LC_{50} (C). Antilog form of Equation (2)

$$C = aW^b \quad (3)$$

resembles the allometric formulae of Huxley (*3*). It supports the idea that the allometric formulae can be used not only to describe a quantitative relationship between body weight and rates of physiological processes or anatomical structures, but also pharmacological or toxicological activities (*10–12*). The usefulness of the allometric Equation (2) as a mode of depicting LD_{50} or LC50 values has been shown over a wide range of various animals (*5*). Nevertheless, the extrapolations are often quite empirical on the basis of analogies and experiences with similar compounds.

The idea of expressing the relationship between a xenobiotic toxic activity and body weight, as a parameter of an animal species, might be comparable with that originating with the hypothesis leading to the formulation of QSAR, i.e., to the formulation of quantitative approaches to biological activity–chemical structure relationships (*13–17*). The ideas on which the QSAR analysis are based suggest that both approaches, i.e., QSAR analysis and the analysis using allometric equations, may be combined for the extrapolation of data on biological tests among compounds and animal species (*11,12*). It means that a quantitatively expressible relationship between xenobiotic toxic activity and "structure" of both the xenobiotic and the animal species can be expected:

$$\log BA_{ij} = k_{ij} + l_{ij} \log X_i \quad (4)$$

$$\log BA_{ij} = a_j + b_i \log Z_j \quad (5)$$

or another form of the equations, where BA_{ij} denotes an activity of a xenobiotic i on a biological object j, X_i a structural characteristic X of the xenobiotic i (e.g., n-octanol/water or oil/air partition coefficient, quantum chemical indices, molecular connectivity, etc.), Z_j a parameter Z of the biological object j (e.g.,, body weight, body surface, a metabolic activity, distribution volumes, etc.) (*11,12*).

This approach may be useful in the extrapolation of data among biological species; the predicted values of xenobiotics from one animal to another are checked by the whole system of formulae connecting a series of xenobiotics with a series of animals. It might reveal outiers caused by disparate metabolism or transport of the xenobiotic or caused by different experimental conditions.

The aim of this paper is to demonstrate the power of the proposed quantitative model: toxicity–chemical structure–biological object for the extrapolation of data among biological objects. For this purpose, LD_{50} values of a series of aliphatic alcohols (C1–C5) obtained with mice, rats, hamsters, guinea pigs, and rabbits have been determined after IP and IV applications and QSARs as well as interspecies correlations have been derived. Values of LD_{50} for rabbit after IP application are estimated and their validity is discussed.

Materials and Methods

Experimental Animals

The animals were taken from a controlled breeding animal farm at the Research Institute for Pharmacy and Biochemistry or from a farm in Velaz: male mice of the strain H, 20–24 g, male rats of the strain Wistar, 200–240 g, male Syrian hamsters, 190–250 g, guinea pigs of both sexes and of various origins, 350–500 g, Chinchilla rabbits of both sexes, 2500–3500 g. The animals had free access to water during the experiment and were fed with a common diet.

Alcohols Applied

Methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, *sec*-butanol and n-pentanol, analytical grade, checked by gas chromatography to have less than 1% impurities, were dosed in aqueous solutions. Higher alcohols, heptanols and octanols, which are poorly soluble in water, were not used for the study because of less reproducible doses as their aqueous suspensions.

Table 1A. Constants used for conversion of LD_{50} values to molar doses.

	MeOH	EtOH	n-PrOH	i-PrOH	n-BuOH	i-BuOH	s-BuOH	n-PenOH
Molecular weight	32.04	46.07	60.10	60.10	74.13	74.13	74.13	88.16
Density, g/mL	0.7914	0.7893	0.8036	0.7864	0.8102	0.8020	0.8063	0.8146

Table 1B. Constants used for conversion from body weight units to body surface units.

	Mouse	Rat	Hamster	Guinea pig	Rabbit
K, m^2/kg [a]	0.2250	0.1780	0.1591	0.1305	0.0376
Body weight, g [b]	22	200	250	400	3500

[a] Data from Spector (*21*).
[b] An average body weight of the species for which the constant K is chosen.

Determination of 50% Lethal Doses (LD_{50})

LD_{50} values were determined from the mortality observed 5 days after an application in one laboratory (Research Institute for Pharmacy and Biochemistry) unless described otherwise. The aqueous solutions of alcohols were used for both IV and IP application. The doses were adjusted by changing the sample volume used, the concentration of the dosing solution remaining constant. Several concentrations (the lowest and the highest ones differed approximately two-fold) of the same alcohol were used to find if there was a dependence of LD_{50} values on the concentration applied.

The LD_{50} values and their 95% confidence intervals were calculated by an approximate graphic probit method (*18–20*). In some cases the number of animals used for the determination was too small for using the graphic probit method. Then, an approximate interval LD_0–LD_{100} was found and LD_{50} taken as an arithmetical mean of the LD_0–LD_{100} interval, which was considered as the 95% confidence interval (guinea pigs, rabbits).

The LD_{50} values determined in mL of 100% alcohol/kg of body weight were converted to mmole/kg or to mmole/m^2 of body surface using the constants given in Table 1.

Pooling of data from individual experiments was carried out using the method of weighted means in cases where no significant differences among them were found by using the χ^2-test (e.g., no dependence of LD_{50} on concentrations applied, etc.)

Statistical Evaluation

An agreement or a difference between the experimental characteristics (LD_{50}) was tested by χ2-test estimating the variances of log LD_{50} from their 95% confidence interval, among regression equations by χ2-test using the estimated covariance matrices of regression coefficients.

The regression equations between experimental characteristics were computed by the weighted least-squares method considering the fact that both variables are due to an error of known quantity (variance of the characteristics). The goodness of fit was tested by χ2-test. When the deviations from a predicted line were significant, the variances of estimated regression coefficients were adjusted by the heterogeneity factor. The significance of regression coefficients was tested by the *t*-test using the adjusted variance.

Molecular Connectivity Indices

Molecular connectivities of the zero order, ${}^0\chi$, and of the first order, ${}^1\chi$, were calculated by a common way proposed by Randič (*22*) and modified by Kier and Hall (*23*) for QSAR analysis:

$${}^0\chi = \sum_r \frac{1}{\sqrt{\delta_r^v}}, \quad {}^1\chi = \sum_{r,s} \frac{1}{\sqrt{\delta_r^v \delta_s^v}}$$

where δ_r^v and δ_s^v are valence atomic connectivities of all atoms r forming an alcohol molecule or of all neighboring atoms *r* and *s* in the molecule (i.e., overall bonds in the molecule), their values being 1 for C(H3), 2 for C(H2), 3 for C(H) and 5 for O(H) (Table 2).

Table 2. Molecular connectivities used in the QSAR analysis of LD_{50} after intraperitoneal application of a series of aliphatic alcohols.

Alcohol	Molecular connectivity ${}^0\chi$	${}^1\chi$
MeOH	1.447	0.447
EtOH	2.154	1.023
n-PrOH	2.861	1.523
i-PrOH	3.024	1.412
n-BuOH	3.568	2.023
i-BuOH	3.731	1.878
s-BuOH	3.731	1.950
n-PenOH	4.275	2.523

Table 3. Primary experimental values of LD_{50} of a series of aliphatic alcohols after IP application and the estimated values.[a]

Alcohol		LD_{50}, mmole/kg: Mouse	Rat	Hamster	Guinea pig[b]	Rabbit
MeOH	Exptl	336(299,373)	237(222,252)	267(235,304)	111(74.1,148)	—
	Estd	353(297,421)	235(217,254)	321(254,406)	187(86.8,404)	57.0(51.1,63.5)
EtOH	Exptl	137(120,154)	81.4(72.7,89.9)	129(120,137)	137(103,171)	—
	Estd	131(110,156)	86.9(80.3,93.9)	110(87.0,139)	74.1(34.3,160)	20.9(18.4,23.8)
n-PrOH	Exptl	61.5(46.1,76.9)	37.4(34.8,40.1)	38.9(36.2,41.6)	20.1(13.4,26.7)	—
	Estd	52.0(43.6,61.9)	36.0(33.3,38.8)	41.5(32.8,52.5)	32.1(14.9,69.3)	8.57(6.43,10.2)
i-PrOH	Exptl	81.1(65.4,96.8)	47.1(33.9,60.2)	57.7(47.2,68.2)	—	—
	Estd	74.5(63.0,89.3)	45.5(42.0,49.2)	57.3(45.4,72.4)	42.6(19.8,92.1)	11.1(9.51,12.9)
i-BuOH	Exptl	—	9.71(5.94,13.5)	—	—	—
	Estd	24.3(20.4,29.0)	18.9(15.8,22.8)*	18.9(15.0,23.7)	16.2(7.50,35.0)	4.36(3.80,5.00)
s-BuOH	Exptl	—	—	—	—	—
	Estd	20.6(16.8,25.4)	16.1(9.27,27.8)	16.4(13.0,20.8)	14.4(6.66,30.9)	3.74(2.44,5.72)
n-PenOH	Exptl	—	6.70[c]	—	—	—
	Estd	11.0(9.27,13.2)	6.57(6.27,7.03)	7.10(5.00,10.1)	6.98(3.24,15.0)	1.59(1.32,1.91)

[a] A weighted mean and its 95% confidence interval; the experimental values are the first line of each pair, the estimated ones, the second line.
[b] Values of LD_{50} obtained from a comparatively small group of animals (4–6 animals for a dose).
[c] Estimated value from LD_{lowest} (*24*): $LD_{50} = 1.2\ LD_{lowest}$.
* *Statistically significant difference between the experimental and the estimated values at $p < 0.05$.*

Table 4. Experimental and the estimated values of LD_{50} of a series of aliphatic alcohols after IP application.[a]

Alcohol		LD_{50}, mmole/m² Mouse	Rat	Hamster	Guinea pig	χ^2-test[b]	LD_{50}, mmole/m², rabbit[c]
MeOH	Exptl	1493(1329,1658)	1489(1397,1583)	1499(1319,1707)	851(568,1136)	7.739	1474(1360,1598)
	Estd	1571(1391,1871)	1474(1363,1594)	1803(1426,2280)	1434(665,3094)	13.339	1515(1360,1688)
EtOH	Exptl	609(533,684)	512(457,565)	723(675,771)	1050(788,1313)	43.581	660(545,799
	Estd	583(489,694)	546(505,590)	618(489,782)	568(263,1226)	4.982	557(490,634)
n-PrOH	Exptl	273(205,342)	235(219,252)	219(103,234)	154(102,205)	8,677	227(208,246)
	Estd	231(194,275)	226(184,295)	233(184,295)	246(114,531)	7,566	228(171,272
i-PrOH	Exptl	360(291,378)	296(213,378)	324(265,383)	—	1.376	332(306,361)
	Estd	331(280,397)	286(264,309)	322(255,407)	327(152,706)	10.417	295(253,344)
n-BuOH	Exptl	77.8(63.1,92.4	89.3(68.5,110)	66.3(48.3,78.4)	92.1(58.6,126)	3,692	78.8(69.0,90.1)
	Estd	99.0(83.1,118)	94.5(87.4,102)	87.7(56.2,137)	106(49.1,229)	2.089	94.6(72.8,125)
i-BuOH	Exptl	—	61.0(26.1,84.9)	—	—	—	—
	Estd	108(90.7,129)	119(99.0,143)*	106(84.4,133)	124(57.5,268)	29,474	116(101,133)
s-BuOH	Exptl	—	—	—	—	—	—
	Estd	91.7(74.5,113	101(58.3,175)	92.2(72.9,117)	110(51,0,237	185,631	99.4(65.0,152)
n-PenOH	Exptl	—	42.1(33.4,53.0)[d]	—	—	—	—
	Estd	49.1(41.2,58.5)	41.3(39.4,44.2)	39.3(28.1,56.6)	53.5(24.8,115)	38.966	42.3(35.2,50.9)

[a] A weighted mean and its 95% confidence interval. The values are calculated from the original values expressed in mmole/kg presented in Table 3.

[b] Values of χ^2-test of a difference among the values of LD_{50} of the four species investigated.

[c] Estimated values; the higher values are estimated from the experimental LD_{50} values of mouse, rat, hamster, and guinea pig as a weighted mean; the lower values from the values estimated for the four species.

[d] Estimated from value of $LD_{lowest}(22)-LD_{50} = 1.2\ LD_{lowest}$.

*Statistically significant difference between the experimental and the estimated values at $p < 0.05$.

Results

The primary set of experimental LD_{50} values (mmole/kg) of aliphatic alcohols C1–C5 after IP and IV applications are summarized in Tables 3 (the first line of data, IP) and 5 (the first line of data, IV). The LD_{50} values converted to body surface (mmole/m²) are presented in Tables 4 (the first line of data, IP) and 5 (the second line of data, IV). No dependence of LD_{50} values on a concentration applied has been found; therefore all observations were included in the calculation regardless of the concentration (30–50 animals for one dose in the case of mice and rats, 10–20 for hamsters). Figure 1 qualitatively demonstrates a dependence of LD_{50} values (mmole/m², IP) on length of alkyl chains in alcohols.

In the next step we have completed the matrix of LD_{50} values after IP application (where more data than after IV application have been collected) with data estimated using methods of QSAR analysis or allometric equations.

A statistically significant correlation was found between LD_{50} values (mmole/m²) obtained after IV application and those after IP application with mice and rats (Table 6) and between LD_{50} values (mmole/m²,IP) and molecular connectivity index of the first order $^1\chi$ of the alcohols (Table 6). No correlation was found with the zero-order molecular connectivity $^0\chi$. The log LD_{50}–$^1\chi$ correlation for guinea pig was less significant because of a large 95% confidence interval due to the small number of animals used for determining LD_{50}. The LD_{50} values obtained after IP application showed interspecies correlations among the animal under study (i.e., mouse, rat, hamster, and guinea pig) being least significant in the case of guinea pig (Table 7).

Three LD_{50} estimates (mmole/m²,IP) were obtained for each alcohol and animal in the matrix using LD_{50} IV–LD_{50} IP intercorrelations, interspecies correlations (especially with LD_{50} of mouse and rat) and correlations with molecular connectivity $^1\chi$. As no statistically significant difference were found among those three esti-

Table 5. Experimental values of LD_{50} of a series of aliphatic alcohols after their IV application.[a]

Alcohol	LD_{50} Mouse	Rat	Rabbit[b]
MeOH	147(126,171)	66.5(61.5,71.2)	278(185,371)
	653(560,760)	418(387,448)	7394(4927,9854)
EtOH	48.0(43.9,52.6)	39.5(35.4,43.8)	51.5(34.3,68.5)
	213(195,234)	248(223,275)	1370(911,1823)
n-PrOH	11.6(8.82,15.2)	9.82(7.65,12.5)	8.04(6.69,9.36)
	51.6(39.2,67.7)	61.7(48.1,78.6)	213(178,249)
i-PrOH	25.1(23.9,26.3)	18.1(17.0,19.3)	19.7(16.4,22.9)
	112(106,117)	114(107,121)	522(435,609)
n-BuOH	6.07(5.25,7.76)	4.18(3.64,4.86)	—
	27.0(23.3,34.5)	26.3(22.9,30.5)	—
i-BuOH	5.63(4.33,7.24)	4.59(4.32,4.86)	—
	25.0(19.2,32.2)	28.8(27.2,30.5)	—
s-BuOH	—	1.86(0.98,3.26)	—
	—	11.7(6.16,20.5)	—
n-PenOH	3.23(3.03,3.41)	2.22(1.85,2.67)	—
	14.4(13.5,15.2)	14.0(11.6,16.8)	—

[a] The weighted mean and 95% confidence interval. The first of each pair of lines is LD_{50} values expressed in mmole/kg; the second line is LD_{50} in mmole/m².

[b] The values of LD_{50} obtained from a small group of rabbits (2–3 animals for a dose), for which only an interval LD_0-LD_{100} can be determined. The values of LD_{50} are taken as the arithmetical mean.

mates for the individual cases, they were included in one weighted average with its estimated 95% confidence interval (Table 4, the second line of data and Fig. 1). Their values given in mmole/kg of body weight are summarized in Table 3 (the second line of data).

No significant difference was found between the experimental LD_{50} values and those estimated by the way described above with the only exception of *i*-BuOH for rat. Even the estimates of LD_{50} values (mmole/m^2, IP) of MeOH for guinea pig were satisfactory because of a wide 95% confidence interval for the estimates.

Statistically highly significant agreement was found among experimental LD_{50} values of individual alcohols expressed in mmole/m^2 units after IP application for all four animal species (Table 4, the column χ^2-test). Such agreement among LD_{50} values was not found if they were expressed in mmole/kg units (Table 3) or after IV application (Table 5). The LD_{50} values for rabbit (mmole/m^2,IP) were, thus, estimated as weighted means of the experimental LD_{50} values (Table 4, the first line in the column, "Rabbit") or of the estimated ones (Table 4, the second line in the column, "Rabbit") for mouse, rat, hamster, and guinea pig. In Table 3 containing the primary set of experimental LD_{50} values, the estimates for rabbit are given as their averages.

Discussion

The results summarized in Tables 3, 4, 6, and 7 support the suggestion that QSAR analysis can be helpful in an extrapolation of toxic indices among various animal species. Several ways for extrapolation of LD_{50} values of aliphatic alcohols after IP application have been followed: use of a similarity between regression equations describing a relation between log LD_{50} (mmole/m^2) and molecular connectivity $^1\chi$ after both types of application used (IP and IV) for animals studied (mouse, rat, and hamster); use of a similarity of intercorrelations between LD_{50} values of various species after IP and IV applications; to employ LD_{50} after IV application using intercorrelations between LD_{50} values obtained after IV and IP applications; to employ allometric equations, i.e., to find a relation between log LD_{50} (IP) and a characteristic parameter of animal species.

Tables 7 and 8 show a similarity between the regression equations describing intercorrelations between LD_{50} values for mouse and rat after IP and IV applications.

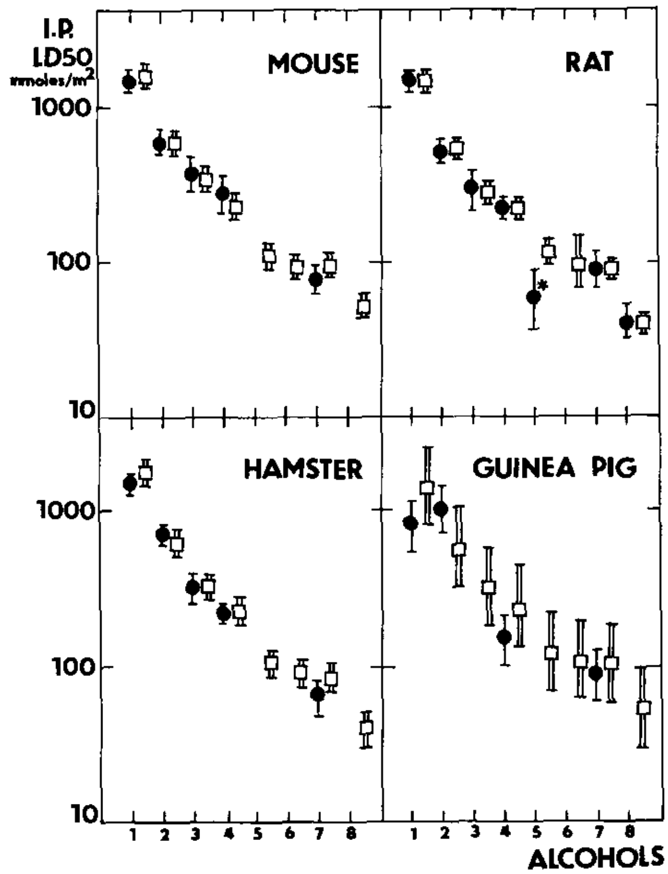

FIGURE 1. Semilog plot of LD_{50} values (mmole/m^2) after IP application for individual aliphatic alcohols. Comparison among the animal species under study. The experimental (●) and estimated (□) values (Table 4) are plotted for individual aliphatic alcohols: (1) methanol, (2) ethanol, (3) isopropanol, (4) *n*-propanol, (5) isobutanol, (6) *sec*-butanol; (7) *n*-butanol, (8) *n*-pentanol (the alcohols are arranged according to increasing length of their carbon chains). The short vertical abscissas represent 95% confidence interval of the data.

Table 6. Constants of regression equations correlating log LD_{50}(mmole/m^2) after IP application with log LD_{50} (mmole/m^2) after IV application or with the first-order molecular connectivity $^1\chi$.[a]

$y = bx + a$								
y	x	b	a	n	SD	χ^2	t_b	Species
log LD_{50} (IP)	log LD_{50} (IV)	0.834 ± 0.113	0.840 ± 0.254	6	0.073	56.57	11.115	Mouse
		1.056 ± 0.130	0.403 ± 0.094	5	0.074	64.25	10.922	Rat
	$^1\chi$	−0.768 ± 0.118	3.546 ± 0.135	5	0.071	89.35	−10.129	Mouse
		−0.751 ± 0.032	3.505 ± 0.035	5	0.018	15.34	−37.279	Rat
		−0.863 ± 0.152	3.683 ± 0.239	5	0.083	313.21	−6.714	Hamster
		−0.736 ± 0.592	3.508 ± 0.766	4	0.278	327.41	−1.964[b]	Guinea pig
log LD_{50} (IV)	$^1\chi$	−0.795 ± 0.057	3.157 ± 0.102	6	0.046	162.12	−21.274	Mouse
		−0.808 ± 0.152	3.072 ± 0.231	8	0.140	157.63	−8.119	Rat
		−1.381 ± 0.470	4.543 ± 0.642	4	0.184	255.25	−3.377	Rabbit

[a]The constants are given ± 1.96 SE corrected for the value of χ^2-test. n is the number of data pairs in the correlation; SD is the standard deviation of the estimate; χ^2 values of χ^2-test, t_b values of t-test of the regression coefficient b.
[b]Not significant.

Table 7. Matrix of constants of regression equations correlating log LD_{50} (mmole/m^2) values obtained after IP application for the animal species studied.[a]

	Mouse	Rat	Hamster	Guinea pig
Mouse		0.981 ± 0.131	0.908 ± 0.140	0.855 ± 0.543
		0.087 ± 0.371	0.245 ± 0.392	0.385 ± 1.466
		44.271 5	48.626 5	213.138 5
		0.062 10.45	0.068 9.383	0.215 2.346
Rat	1.006 ± 0.134		0.934 ± 0.277	0.753 ± 0.575
	-0.050 ± 0.384		0.097 ± 0.805	0.642 ± 1.546
	45.402 5		153.146 5	255.169 5
	0.062 10.35		0.117 4.858	0.270 2.049[b]
Hamster	1.081 ± 0.168	0.995 ± 0.294		0.928 ± 0.458
	-0.215 ± 0.467	0.083 ± 0.8333		0.193 ± 1.234
	57.263 5	160.611 5		160.248 5
	0.080 8.566	0.128 4.698	0.900 ± 0.438	0.237 2.909
Guinea pig	0.902 ± 0.572	0.926 ± 0.688	0.900 ± 0.438	
	0.263 ± 1.538	0.218 ± 1.824	0.266 ± 1.178	
	261.061 5	401.820 5	180.900 5	
	0.288 2.293[b]	0.323 1.936[b]	0.230 2.991	

[a]For each block of values the first line is the regression coefficient b ± 1.96 SE corrected for the value of χ^2- test; the second line is the constant a ± 1.96 SE corrected for the value of χ^2-test of the regression equation $y = bx + a^c$, the first value on the third line is the χ^2-test; the second value in the third line is the number of data pairs; The first value on the fourth line is the SD of the estimate, and the second value in the fourth line is the t-test of the regression coefficient b.
[b]Not significant.

If one tries to apply this fact for the intercorrelations between LD_{50} values of rabbit and mouse or of rabbit and rat (Table 8), estimates of LD_{50} (mmole/m^2, IP) for rabbit were too high, e.g., as high as about 40 mL/kg of MeOH or 5 to 6 mL/kg of n-PrOH. Table 6 indicates a similarity between the regression equations describing the relationships between log LD_{50} (mmole/m^2) and molecular connectivity $^1\chi$ after IP and IV applications for mouse and rat, the constant a being higher by about 0.4 log units in the case of IP application,. Applying this to the rabbit (after IV application, the last line of Table 6) leads again to unreal estimates (30–35 mL/kg for MeOH or about 2 mL/kg for n-PrOH). Thus, we have found no way to extrapolate LD_{50} values for rabbit obtained after IV application to estimate those after IP application, although a close correlation between these two types of LD_{50} values exists in the case of mouse and rat (Table 6, the first two lines) and undoubtedly exists even for rabbit. This might be explained by differences in transformation or distribution processes in these three species (mouse, rat, and rabbit) after IP and IV applications of an alcohol.

Another striking similarity exists among LD_{50} values of each of the alcohols studied for all animal species chosen if they are expressed in mmole/m^2 units (Table 4). No significant difference can be found among the LD_{50} values of any of the alcohols for mouse, rat, and hamster. Those in guinea pig sometimes show differences, but their wide 95% confidence interval makes them comparable with the others. By using a weighted mean as a prediction for rabbit (which virtually simulates an allometric equation), LD_{50} values of about 2.3 mL/kg for MeOH or about 0.6 mL/kg for n-PrOH are obtained, which are much more reasonable than the

Table 8. Matrix of constants of regression equations correlating log LD_{50} (mmole/m^2) obtained after IV application for the species studies.[a]

	Mouse	Rat	Rabbit
Mouse		0.960 ± 0.065	0.693 ± 0.069
		0.057 ± 0.128	0.152 ± 0.202
		26.789 6	5.493 4
		0.033 21.05	0.026 16.10
Rat	1.036 ± 0.070		0.529 ± 0.154
	−0.050 ± 0.137		0.623 ± 0.460
	28.850 6		41.418 4
	0.036 20.24		0.060 5.937
Rabbit	1.436 ± 0.144	1.805 ± 0.526	
	−0.203 ± 0.311	−0.991 ± 1.153	
	11.345 4	140.472 4	
	0.052 11.18	0.200 3.259	

[a]See footnote to Table 7.

predictions mentioned above.

Let us continue to define a quantitative relationship between LD_{50} (mmole/m^2, IP) and a parameter of the animal species chosen [Eq. (5)]. The type of LD_{50} values used is independent of the animal tested, but dependent on the chemical structure of the alcohol. Therefore the species parameter may be arbitrarily chosen, e.g., body weight, body surface or their ratio. We have chosen the log form of the body surface: body weight ratio (unpublished results). The regression equations

$$\log LD_{50}\ (\text{mmole/m}^2,\ \text{IP}) = f(\log \text{body surface: body weight})$$

have a regression coefficient of about zero and an intercept with the LD_{50} axis that is close to the estimates given in Table 4.

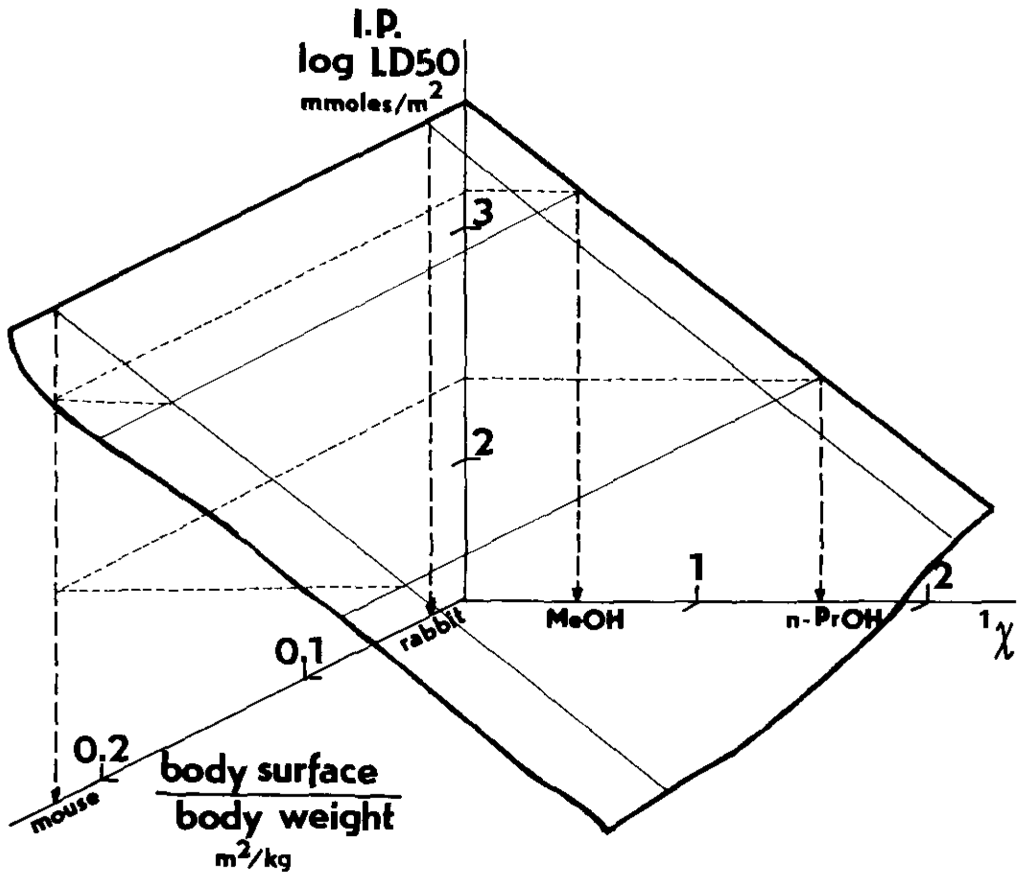

FIGURE 2. Graphic representation of the quantitative structure-activity-species relationships for the system: log LD_{50} (mmole/m^2, IP)–aliphatic alcohols (C_1-C_5)–mammals (mouse, rat, hamster, guinea pig, rabbit). The intercept of the model plane with the xy-plane is described by the line representing a regression equation of a dependence of log LD_{50} on $^1\chi$ common to mouse, rat, hamster, guinea pig (and rabbit): log LD_{50} = -0.78 $^1\chi$ + 3.56, that with the yz-plane by the line parallel to the z-axis at y = 3.56.

Figure 2 schematically illustrates the situation showing a plot of log LD_{50} (mmole/m^2, IP) against both molecular connectivity $^1\chi$ (parameter of the chemical structure) and body surface: body weight ratio (parameter of animal species). It is represented by a plane that intersects the LD_{50}–body surface: body weight plane in a line parallel to the body surface: body weight axis and the log LD_{50}–$^1\chi$ plane in a line described by the regression equation log LD_{50} = f ($^1\chi$) (Table 6).

Using the hypothesis published earlier (*11,12*) (Eqs. 4 and 5), it is possible to conclude from the study of this system of alcohols, animals and LD_{50} (mmole/m^2, IP) that: the constant b_i is not dependent on chemical structure of alcohols, being close to zero; the constant a_i is a linear function of molecular connectivity $^1\chi$ (close to -0.78 $^1\chi$ + 3.56); the constants l_j and k_j are not dependent on the parameter used for the description of animal species, i.e., body surface: body weight ratio being l_j = -0.78, k_j = 3.56.

This rather simple example points out advantages of the QSASR hypothesis suggested earlier (*11,12*), but a large number of difficultly obtainable experimental results necessary for a construction of the model remains, however, an unpleasant disadvantage. A determination of additional LD_{50} values is necessary to prove that the model is valid in the whole scale of the system chosen.

This study also indicates that the expression of the magnitude of toxic effects in units of mmole/m^2 might often be more helpful than that expressed in mmole/kg units.

REFERENCES

1. Günther, B. Dimensional analysis and theory of biological similarity. Physiol. Rev. 55: 659–699 (1975).
2. Günther, B., and Morgado, E. Theory of biological similarity revised. J. Theor. Biol. 96: 543–559 (1982).
3. Huxley, J. S. Problems of Relative Growth. Methuen, London, 1932.
4. Gould, S. J. Allometry and size in ontogeny and phylogeny. Biol. Rev. 41: 587–640 (1966).
5. Anderson, P. D., and Weber, L. J. Toxic responses as a quantitative function of body size. Toxicol. Appl. Pharmacol. 33: 471–483 (1975).
6. Pallotta, A. J., Kelly, M. G., Rall, D. P., and Ward, J. W. Toxicology of acetoxycycloheximide as a function of sex and body weight. J. Pharmacol. Exptl. Therap. 136: 400–405 (1962).
7. Lamanna, C., and Hart, E. R. Relationship of lethal dose to body weight of mouse. Toxicol. Appl. Pharmacol. 13: 307–315 (1968).
8. Freireich, E. J., Gehan, E. A., Rall, D. P., Schmidt, L. H., and Skipper, H. E. Quantitative comparison of toxicity of anti-cancer

agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemother. Repts. 50: 219–244 (1966).
9. Bliss, C. I. The size factor in the action of arsenic upon silkworm larvae. J. Exptl. Biol. 13: 95–110 (1936).
10. Trčka, V. Attempts in data extrapolations between various animals and man (in Czech). Čas. Lék. Čes. 121: 1062–1065 (1982).
11. Tichý, M., and Trčka, V. Contribution of QSAR analysis to data extrapolation between biological objects. In: Quantitative Approaches to Drug Design (J. C. Dearden, Ed.), Elsevier Science Publishers, Amsterdam, 1983, pp. 33–41.
12. Tichý, M., and Trčka, V. A quantitative model: activity–biological object–chemical structure (in Czech). Čas. Lék. Čes. 122: 936–939 (1983).
13. Purcell, W. P., Bass, G. E., and Clayton, M. J. Strategy in Drug Design. Wiley-Interscience Publishers, New York, 1973.
14. Martin, Y. C. Quantitative Drug Design. Marcel Dekker, New York–Basel, 1978.
15. Seydel, J. K., and Schaper, K. J. Chemische Struktur and biologische Aktivität von Wirkstoffen. Verlag Chemie, Weinheim, 1979.
16. Hansch, C., and Leo, A. J. Substituent Constants for Correlation Analysis in Chemistry and Biology. Wiley-Interscience Publishers, New York-Chichester-Brisbane-Toronto, 1979.
17. Franke, R. Optimierungs Methoden in der Wirkstofforschung-Quantitative Struktur-Wirkungs-Analyse. Akademie Verlag, Berlin, 1980.
18. Litchfield, J. T., and Wilcox, F. W. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 95: 99 (1949).
19. Roth, Z. A graphic probit method for a calculation of LD_{50} and relative toxicity (in Czech). Čs. Fysiol. 10: 408 (1961).
20. Finney, D. J. Statistical Methods in Biological Assay. Charles Griffin, London, 1964.
21. Spector, W. S. Handbook of Biological Data. Saunders, Philadelphia-London, 1956.
22. Randić, M. On characterization of molecular branching. J. Am. Chem. Soc. 97: 6609–6615 (1975).
23. Kier, L. B., and Hall, L. H. Molecular Connectivity in Chemistry and Drug Research. Academic Press, New York-London, 1976.
24. Registry of Toxic Effects of Chemical Substances (R. J. Lewis, Ed.), National Institute for Occupational Safety and Health, Cincinnati, 1979.